Am. J. Hum. Genet. 60:842–850, 1997

The Prevalence and Wide Clinical Spectrum of the Spinocerebellar Ataxia Type 2 Trinucleotide Repeat in Patients with Autosomal Dominant Cerebellar Ataxia

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Summary

The dominant cerebellar ataxias (ADCAs) represent a clinically and genetically heterogeneous group of disorders linked by progressive deterioration in balance and coordination. The utility of genetic classification of the ADCAs has been highlighted by the striking variability in clinical phenotype observed within families and the overlap in clinical phenotype observed between those with different genotypes. The recent demonstration that spinocerebellar ataxia type 2 (SCA2) is caused by a CAG repeat expansion within the ataxin-2 gene has allowed us to determine the frequency of SCA2 compared with SCA1, SCA3/Machado-Joseph disease (MJD), and dentatorubropallidoluysian atrophy (DRPLA) in patients with sporadic and inherited ataxia. SCA2 accounts for 13% of patients with ADCA (without retinal degeneration), intermediate between SCA1 and SCA3/MJD, which account for 6% and 23%, respectively. Together, SCA1, SCA2, and SCA3/MJD constitute >40% of the mutations leading to ADCA I in our population. No patient without a family history of ataxia, or with a pure cerebellar or spastic syndrome, tested positive for SCA1, SCA2, or SCA3. No overlap in ataxin-2 allele size between normal and disease chromosomes, or intermediate-sized alleles, were observed. Repeat length correlated inversely with age at onset, accounting for ~80% of the variability in onset age. Haplotype analysis provided no evidence for a single founder chromosome, and diverse ethnic origins were observed among SCA2 kindreds. In addition, a wide spectrum of clinical phenotypes was observed among SCA2 patients, including typical mild dominant ataxia, the MJD phenotype with facial fasciculations and lid retraction, and early-onset ataxia with a rapid course, chorea, and dementia.

Received November 5, 1996; accepted for publication January 7, 1997.

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0002-9297/97/6004-0013$02.00

Introduction

The dominantly inherited spinocerebellar ataxias (ADCAs) are a group of genetically diverse neurological conditions that share progressive deterioration in balance and coordination due to degeneration of the cerebellum and its afferent and efferent pathways (Harding 1993; Rosenberg 1990, 1995). Extracerebellar deterioration occurs in most patients as well, presenting as variable nuclear or supranuclear opthalmoparesis, slow saccades, pyramidal or extrapyramidal signs, neuropathy, or decreased vibration sense. Moderate or severe dementia is an unusual feature (Harding 1982, 1993; Durr et al. 1993). Typical onset of symptoms occurs between the ages 30 and 40 years, and symptoms are slowly progressive, but, in successive generations, onset may be earlier and progression more severe, a phenomenon known as "anticipation." Harding (1982) provided a useful and enduring classification of the ADCAs by separating dominant ataxias into ADCA I (without retinal degeneration), ADCA II (with retinal degeneration), and ADCA III (pure cerebellar). ADCA I is the most prevalent of these conditions.

More refined clinical classification of the ADCAs has been hampered by the marked variation in phenotypes that is observed even within families. ADCA I kindreds with remarkably different clinical features have been shown to carry the same disease gene, while some kindreds that are clinically indistinguishable have mutations at different loci (Durr et al. 1993; Cancel et al. 1995*a*, 1995*b*; Dubourg et al. 1995; Lezin et al. 1996). At least six genetic loci have been identified, and two, spinocerebellar ataxias (SCA), SCA1 and SCA3/Machado-Joseph disease (MJD), have previously been shown to be due to expansions of a CAG nucleotide repeat (Gispert et al. 1993; Orr et al 1993; Banfi et al. 1994; Ranum et al. 1994*b*; Twells et al. 1994; Benomar et al. 1995; Giunti et al. 1995; Flanigan et al. 1996; Higgins et al. 1996). Expansion of a CAG repeat has also been demonstrated in dentatorubropallidoluysian atrophy (DRPLA), which can present with an SCA phe-

notype, but is rare outside of Japan (Nagafuchi et al. 1994). The role of CAG repeats in degenerative neurological conditions has been further highlighted by the recent cloning of the gene for SCA2 (Imbert et al. 1996; Pulst et al. 1996; Sanpei et al 1996), which also carries an expanded CAG repeat on disease chromosomes and the detection of a polyglutamine expansion in patients with SCA7 (Lindblad et al. 1996).

The widespread availability of direct gene testing for SCA1 and SCA3/MJD has permitted the determination of the frequency of SCA1 and SCA3/MJD in several large populations. In some ethnic groups, the SCA1 and SCA3 mutations account for a large percentage of the ataxic population (Sasaki 1993; Giunti et al. 1994; Schols et al. 1995; Silveira et al. 1996). However, in many ethnically diverse populations present in large cities in North America, India, South America, and Europe, SCA1 and SCA3 together account for <30% of patients with dominant ataxia (Ranum et al. 1995; Silveira et al. 1996). Therefore, identification of the mutational basis of the remaining 70% of patients remains an important goal of current research (Rosenberg 1990, 1995).

The recent identification of the SCA2 expanded repeat by members of our group offered the opportunity to test the frequency of SCA2 compared with SCA1, SCA3, and DRPLA in a large population of patients with inherited or sporadic ataxia and to highlight important clinical features of SCA2. We have also been able to verify the range of the repeat sizes found on normal and disease chromosomes. Since previously described SCA2 kindreds were identified on the basis of linkage analysis, these studies, while comprehensive, were naturally biased toward large kindreds with living members in several generations (Orozco-Diaz 1992; Pulst et al. 1993; Sasaki 1993; Cancel et al. 1995*b*; Durr et al. 1995*a*, 1995*b*). Thus, kindreds with less severe disease and slower progression may have been studied in some cases. The identification of the CAG expansion has also made it possible for us to identify and study the clinical features of patients in several kindreds with a wide range of disease phenotypes selected without these earlier biases.

Methods

Patient Identification

Eighty-one individuals from 47 kindreds with ADCA I (according to Harding's [1982] classification) were available for genetic testing from among 65 families followed in the ataxia clinic at the University of California, Los Angeles (UCLA), Neurological Services (examined by S.P., D.H.G., and others with experience in evaluating patients with ataxia). These individuals represent an unselected sample from patients followed at UCLA with ADCA I. (The family used for mapping SCA2 was omitted from the frequency analysis but included in the haplotype analysis.) Four of the MJD patients had previously been tested, and one of the SCA1 patients was included in a previous series as well (Ranum et al. 1995). This clinic is a referral center for a large region of central and southern California and Nevada. Since many Californians have moved from other locations in the past 25 years, the clinic population is ethnically and geographically diverse.

Clinical information was obtained by detailed chart review (D.H.G.). Examinations at many points in time were usually available for review, but the analysis of salient clinical features focused on examinations done between 5 and 10 years into the illness. In one pedigree, where the disease led to death within 10–15 years, exams performed within the first 5 years were used. Progressive cerebellar ataxia and dysarthria were present in all cases. Slow saccades, saccadic pursuit, and opthalmoparesis were present in a majority. Other features such as pyramidal signs, basal ganglia signs or myoclonus, peripheral neuropathy, optic atrophy, and decreased vibration sense were variably present. Subjects were included without regard to ethnicity, age at onset, or severity of symptoms.

Nineteen additional individuals with either a pure cerebellar or spastic phenotype were also tested, as were 29 individuals with sporadic SCA and 6 patients with a possible recessive mode of transmission. Dominant inheritance was presumed on the basis of having at least one family member affected in two or more successive generations and vertical parent-to-child transmission. Sporadic occurrence was determined on the basis of a negative family history. Recessive inheritance was suspected on the basis of a history of consanguinity or having two siblings affected without symptomatic parents. At least one symptomatic individual from each family underwent genetic testing. Age at onset was determined in the majority of the affected individuals from five of the six SCA2 kindreds ($N = 30$) by clinical history from the patient or other available family members.

Screening for known reversible or nonreversible forms of ataxia included complete blood count with differential, electrolytes, Westergren erythrocyte sedimentation rate, antinuclear antibody, pyruvate, lactate, liver-function testing, thyroid-function tests, vitamins E, B1, and B12, phytanic acid, lysosomal screen, very-long-chain fatty acids, urine for heavy metals, and cranial imaging. Additional diagnostic testing varied according to the clinical symptoms at presentation. Human T-cell lymphotrophic virus-I was investigated in patients with spastic paraparesis, and paraneoplastic antipurkinje cell antibody screening was done in a majority of sporadic patients. Neuropthalmologic examinations, electroretinograms, and electronystagnograms were pursued when clinically appropriate.

Molecular Genetic Analysis

SCA repeat analysis.—DNA was isolated from peripheral leukocytes or lymphoblastoid cell lines as described by Nechiporuk et al. (1996). SCA1 and SCA3/MJD alleles were amplified and analyzed on agarose and acrylamide gels by standard methods (Orr et al. 1993; Sutton and Pulst, in press). For SCA3/MJD, primer pairs MJD52 and the novel primer MJDB 5′-GTAACCTTG-CTCCTTAATCC-3′ were used (Sutton and Pulst, in press). DRPLA alleles were amplified using primers and conditions described by Burke et al. (1994). Several patients with a MJD phenotype who tested negative for SCA3 in our lab were confirmed by testing for SCA1 and SCA3 in an outside DNA diagnostic laboratory (Baylor College of Medicine).

For SCA2 allele analysis, 1 μM each of primers SCA2-A (5′-GGGCCCC TCACCATGTCG-3′) and SCA2-B (5′-CGGGCTTGCGGACATTGG-3′) were added to 20-40 ng human genomic DNA with standard buffer and nucleotide concentrations in a final volume of 20 μl (Pulst et al. 1996). After an initial 5-min denaturation at 95°C, 35 cycles of 96°C denaturation (90 s), 63°C annealing (30 s), and 72°C extension (90 s), followed by a final extension of 72°C for 5 min, were performed. Expanded alleles were reamplified using ^{32}P-end-labeled primer SCA2-A and separated by electrophoresis through 6% polyacrylamide sequencing gels and analyzed as described by Pulst et al. (1996).

Genotyping and haplotype analysis.—To determine whether a common ancestral chromosome may have carried the SCA2 expansion, haplotypes were generated using polymorphic STR markers D12S1672 and D12S1333. D12S1333 is 200 kb telomeric and D12S1672 is 20 kb centromeric to the SCA2 triplet repeat (Pulst et al. 1996; A. Nechiporuk and S.-M. Pulst, unpublished data). PCR and electrophoresis were conducted as described elsewhere (Nechiporuk et al. 1996; Pulst et al. 1996). No common alleles were detected with these two closely flanking markers (see Results).

Statistical Analysis

To demonstrate the correlation between repeat length and age at onset, values obtained from 15 patients in whom accurate information on age at onset was available were plotted using the Secant method in the Statistical Analysis Software (SAS). The data fit an exponential model (Pulst et al. 1996), Age = 815 (±338 SE) × EXP (−.078 × repeat length), with a mean square residual of 31.8.

Results

Frequency of the SCA2 Expanded Allele

Individuals from 13% (6/47) of kindreds with ADCA I tested positive for the SCA2 expanded CAG repeat.

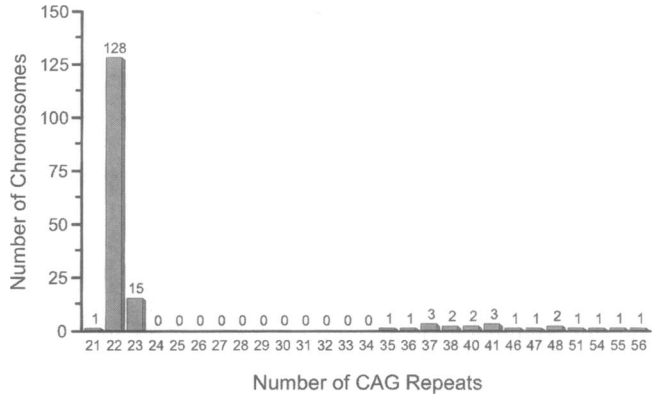

Figure 1 Distribution of normal and pathological SCA2 alleles. Normal alleles range between 21 and 23, while pathological expanded alleles range between 35 and 56. Expanded alleles are graphed discontinuously. The number of chromosomes with each given repeat size is indicated over each bar.

All of the normal alleles were between 21 and 23 repeats, while the expanded alleles ranged from 35 to 56 repeats (fig. 1). No alleles intermediate between 23 and 35 repeats were observed. Thus, there was significant distance between the size of the normal and disease alleles, similar to SCA3/MJD and in contrast to SCA1. In addition, the wide range of normal allele sizes demonstrated in SCA1 and SCA3/MJD were not observed in SCA2. Only three alleles were observed in our series of normal chromosomes at the SCA2 locus, as compared with SCA3/MJD and SCA1, in which more than a dozen have been reported in each (Cancel et al. 1995*a*; Dubourg et al. 1995; Maciel et al. 1995; Ranum et al. 1995; Watkins et al. 1995). Furthermore, one allele (22 repeats) accounted for 90.3% of the normal alleles in our patients, while the other two accounted for 9% (23 repeats) and 0.7% (21 repeats) of the ~150 normal chromosomes tested (fig. 1).

A diverse group of ethnic origins was observed among those positive for SCA2 including two Mexican American kindreds, two African American kindreds, one Swedish kindred, and one kindred of mixed northern European lineages. None of the 54 patients with pure cerebellar, spastic phenotypes, or recessive or sporadic inheritance tested positive.

Average age at onset for SCA2 was 30 years, with a range of 16–58 years (± 12.5 SD), similar to reports based on linkage analysis (Belal et al. 1994; Durr et al. 1995*a*). Children experienced symptoms 9.2 (SD ± 9.4) years earlier on average than their parents, but the range of anticipation was wide and was skewed by large changes in age at onset in several paternal transmissions (fig. 2). Paternal transmissions led to an average earlier age at onset (15.4 years ± 7.7 SD; n = 7) than maternal transmissions (4.4 years ± 7.6 SD; n

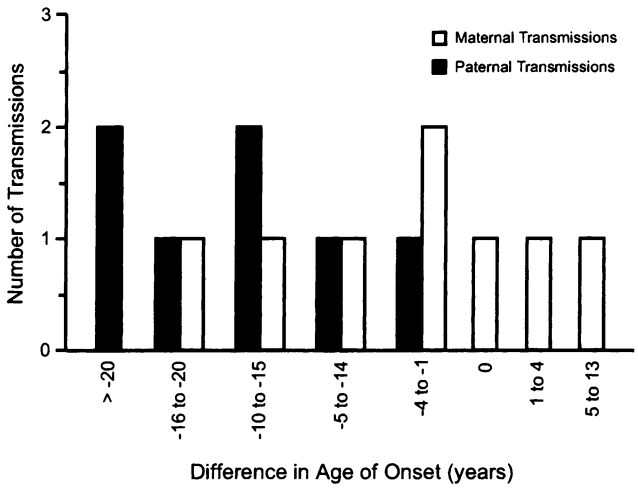

Figure 2 Anticipation in maternal versus paternal transmissions. Difference in age at symptom onset between parents and their children is divided into 5-year intervals (onset age of child − onset age of parent). A decrease in age at onset between successive generations is indicated by negative values, and an increase in age at onset is indicated by positive values. A zero indicates no change in onset age. While the parental transmissions all resulted in a decrease age at symptom onset, maternal transmissions result in a wider distribution in age at onset.

= 9). No paternal transmission resulted in a later age at onset in offspring, while two of eight maternal transmissions resulted in a later age at symptom onset in children than their mother. Individual data are presented graphically in figure 2, to highlight the variability in parent-child age-at-onset differences. In addition, within families the course of the disease appeared to become more rapid and severe in successive generations (not shown), consistent with previous reports (Durr et al. 1995*b*). Too few DNA samples are currently available to determine whether the difference in anticipation between maternal and paternal transmissions or increased severity in successive generations reflects increased instability and expansion of the CAG repeat in paternal transmissions.

There was a significant inverse correlation between repeat size and age at symptom onset (fig. 3). Overall repeat length explained ~80% of the variability in onset age. However, the relationship between repeat length and age at onset was not linear over the entire range of disease allele sizes (fig. 3). Small increments in repeat size had a large effect on the age at onset between 35 and 45 repeats. With repeats >45, the relationship between repeat size and age at onset began to reach an asymptote. After this point, changes in repeat size had relatively less effect on age at onset. Furthermore, those with repeat length between 35 and 45 had onset of symptoms ranging from age 20 to 60 years, while all of those with repeats ⩾45 had onset at <20 years of age.

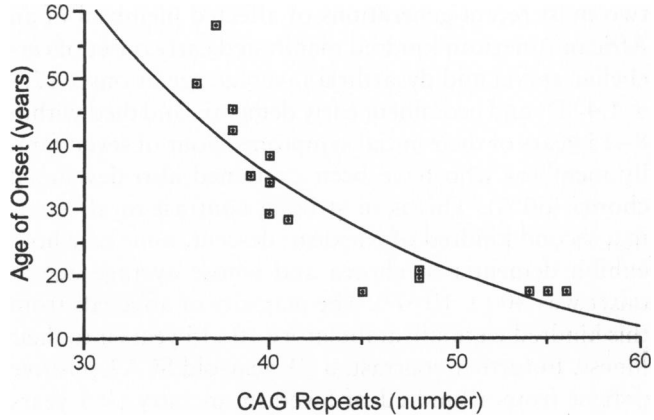

Figure 3 Correlation between age at onset and the number of CAG repeats in SCA2. The data fit a nonlinear model with a correlation coefficient of $r = -.81$ ($n = 15$).

Clinical Correlations

All affected individuals among those that carry the expanded SCA2 repeat shared the classic clinical features of early truncal ataxia and dysarthria, and the majority exhibited slowed saccades (table 1). Pyramidal signs were not a prominent early feature, nor were parkinsonian rigidity or tremor, confirming earlier data based on linkage analysis (Durr et al. 1995*a*). However, chorea and supranuclear opthalmoparesis were observed in individuals in three of six families. Also striking was the early presence of dementia in all symptomatic members of one family, which accounted for all patients with dementia studied.

There was a tendency for clinical phenotypes to be similar within a kindred, while a striking range of features was observed between kindreds. For example, the

Table 1

Clinical Characteristics of Patients with SCA2

Characteristic	Prevalence
Age (years) at onset	30 (± 12.5 SD)
No. of patients (families)	16 (5)
Truncal and gait ataxia	100%
Appendicular ataxia	100%
Slow saccades	92%
Ophthalmoparesis	60%
Neuropathy	44%
Dystonia or chorea	38%
Dementia	37%
Pyramidal signs	31%
Increased tone	7%
Increased tendon reflexes	14%
Babinski sign	12.5%
Fascial fasciculations	23%
Dysphagia	12.5%

two most recent generations of affected members of an African American kindred manifested early onset of cerebellar ataxia and dysarthria (average age at onset 17.7 ± 1.4 SD) and prominent early dementia and died within 8–15 years of their initial symptoms. Four of seven family members who have been examined also developed chorea (60%). This is in striking contrast to affecteds in a second kindred of Mexican descent, none of whom exhibit dementia or chorea and whose average age at onset was 40 (± 10 SD). The majority of affecteds from this kindred were still ambulatory 10–15 years into their illness. In further contrast, a 51-year-old SCA2-positive patient from a kindred of Swedish ancestry (<5 years symptomatic) exhibited a typical MJD phenotype with moderate truncal ataxia, decreased vibration sense, mild dysphagia for liquids, slowed saccades with lid lag, and facial fasciculations. Therefore, a wide spectrum of SCA phenotypes, including ataxia with prominent dementia, MJD, and typical dominant ataxia with a mild course was represented among patients testing positive for the SCA2 CAG expansion.

Frequency of SCA1, SCA3, and DRPLA Expanded Alleles

Three of 47 ADCA I patients were positive for the SCA1 expanded repeat (6%). Repeat length ranged from 40 to 58. One patient was of Greek descent, another Hispanic, and the other a mixture of northern European lineages. Eleven (23%) of 47 ADCA I kindreds were positive for the expanded SCA3/MJD allele. Five were of non-Portuguese northern European ancestry, emanating from Britain, Ireland, Germany, and Sweden. Three were of African American descent, and the three others were of Chinese, Filipino, or Portuguese descent. Therefore, no unusual clustering of ethnicity was found among the three kindreds positive for SCA1 or those carrying the SCA3/MJD mutation. The combined incidence of the SCA1 and SCA3 expanded alleles in our population was 29%, consistent with two recent large series (Ranum et al. 1995; Silveira et al. 1996). No patients with sporadic, cerebellar, or spastic phenotype tested positive for either SCA1 or SCA3. Nor did any of our patients with inherited or sporadic ataxia test positive for the DRPLA expanded repeat.

Of SCA3/MJD-positive patients, only six (55%) carried the pretest diagnosis of probable MJD. These patients either displayed the typical adult-onset type II-III MJD phenotype including opthalmoparesis with bulging eyes, facial fasciculations, ataxia, spasticity, and variable neuropathy or had Portuguese/Azorean ancestry. The other five patients were of mixed ethnicity, including two African Americans and three non-Portuguese northern Europeans. All shared the phenotype of ataxia with slowed saccades and were clinically indistinguishable from patients with other typical ADCAs (ADCA I) such as SCA1 or SCA2.

Table 2

SCA2 Disease Haplotypes

Family	D12S1672	D12S1333
GR (n = 9)	10	9
CO (n = 2)	7	2
MA (n = 15)	9	2
BJ (n = 9)	3	6

NOTE.—The normal alleles have been omitted, and only disease alleles are shown. The number (n) of individuals genotyped is indicated.

Haplotype Analysis

Four of the six SCA2 kindreds contained two or more individuals in whom phase was determined. These individuals were genotyped for two polymorphic markers that closely flank the SCA2 gene. The disease chromosome alleles are displayed in table 2. Each kindred shares a different haplotype on the disease chromosome, suggesting diverse origins for the expansion carrying chromosome.

Discussion

The identification of a CAG repeat within the SCA2 gene has made it possible to determine the frequency with which SCA2 occurs in patients with ADCA, furthering our ability to categorize these patients genetically. In addition to providing for a more definitive diagnosis, the genotype-phenotype correlations now possible are likely to provide important clues that will hasten progress toward the understanding of the pathophysiology of these conditions.

The expanded SCA2 repeat was identified with a frequency of 13% in kindreds with ADCA 1, intermediate between SCA3/MJD and SCA1, which represented 23% and 6% of our population, respectively. This is close to recent estimates of the frequency of SCA2 among French families determined by linkage analysis (14%; Cancel et al. 1995*b*) and by an antibody specific for polyglutamine expansions (18%–24%; Trottier et al. 1995; Stevanin et al. 1996). The prevalence of SCA1 and SCA3/MJD in our population is also within the range that others have reported for SCA1 and SCA3/MJD in large populations of patients with ADCA (Ranum et al. 1995; Higgins et al. 1996; Silveira et al. 1996). Therefore, it is likely that in most ethnically heterogeneous populations, SCA3/MJD will be the most prevalent ADCA, followed closely by SCA2, which is twice as common as SCA1. No patient among those with inherited or sporadic ataxia in our population tested posi-

tive for the DRPLA expanded repeat, supporting the notion that DRPLA is rare outside Japanese populations (Warner et al. 1994; Silveira et al. 1996)

The combined frequency of SCA1, SCA2, and SCA3 in our patients with ADCA I is 42%. In some populations with more homogeneous ethnic origins, these three known mutations are likely to represent an even greater fraction of patients (Sasaki 1993; Schols et al. 1995; Silveira et al. 1996). Therefore, it will now be possible to identify the mutation responsible for disease in a large group of patients with dominant ataxia by use of routine PCR-based testing. The wide variety of phenotypes seen in SCA2 and the significant degree of clinical overlap with SCA1 and SCA3 highlights the difficulty of making a diagnosis on the basis of clinical information alone. This underscores the importance of gene testing for diagnostic accuracy among patients with dominant ataxia (Rosenberg 1995).

We did not detect any de novo cases of SCA2 among 29 individuals without a family history of ataxia in this study. Although more patients with de novo SCA should be tested to determine the rate of spontaneous new SCA2 cases, this data suggests that sporadic cases of SCA2 are likely to be rare, as is the case with SCA1 and SCA3 (Ranum et al. 1995; Higgins et al. 1996; Silveira et al. 1996). Previous testing of 25 patients without family history of ataxia for SCA1, DRPLA, and SCA3 yielded only one patient who was positive for SCA3/MJD whose father died at an early age (Silveira et al. 1996). Furthermore, none of the 19 patients with pure cerebellar or spastic ataxia had the expanded SCA2 allele. Thus, SCA2 gene testing in those without family history or pure cerebellar or spastic phenotypes is likely to have a low yield.

Analysis of SCA2 Alleles

Expanded SCA2 alleles ranged from 35 to 56 repeats, while normal alleles contained between 21 and 23 repeats. The 22-repeat allele appears to be the most common normal allele, accounting for >90% of normal chromosomes in this and other recent analyses (Imbert et al. 1996; Pulst et al. 1996; Sanpei et al. 1996). In addition, the rare 21-repeat normal allele detected in one patient with sporadic ataxia was not detected in previous screening of 110 chromosomes (Pulst et al. 1996) but was detected along with several other rare alleles extending from 15 to 29 repeats in Japanese (Sanpei et al. 1996) and French populations (Imbert et al. 1996). There was no overlap between normal and affected alleles in our population, and no intermediate-sized alleles were found, suggesting that the formation of the expanded disease allele may be a stepwise event rather than a gradual process. This is in striking contrast to SCA1, SCA3, DRPLA, and Huntington disease (HD), where the normal alleles are highly polymorphic (Chung et al. 1993; Orr et al. 1993; Snell et al. 1993; Nagafuchi et al. 1994; Watkins et al. 1995; Maruyama et al. 1996; Rubinsztein et al. 1996). Furthermore, in SCA1 the distribution of disease alleles is closely juxtaposed to normals, and in HD the distribution of disease alleles overlaps with normals (Rubinsztein et al. 1996). This lack of polymorphism in the SCA2 repeat raises the possibility that one or more components of the mechanism for the SCA2 expansion may be different than for the other CAG expansions (Gacy et al. 1995; McMurray 1995).

Repeat size inversely correlated with age at onset and accounted for ~80% ($r = -.81$) of the variability in age at onset, similar to what has been observed in the Japanese SCA2 population (Sanpei et al. 1996) and SCA1 (Ranum et al. 1994*a*). The remaining variance in onset age may be explained by closely linked modifying genes, polymorphisms within the SCA2 gene itself, epigenetic factors, or somatic mosaicism, which has been noted in other CAG repeat conditions (Cancel et al. 1995*a*; Telenius et al. 1995; Ueno et al. 1995).

The correlation between age at onset and repeat size in SCA2 does not appear to be linear. At the lower pathological range of repeat sizes (35–45), small increases in repeat size can have a large effect on age at onset. At larger repeat sizes (>45), similar changes in repeat size exert far less of an effect on age at onset. Furthermore, all patients with >45 repeats have onset of symptoms at ages <20 years. A similar relationship is apparent in the Japanese SCA2 population (although it was originally analyzed as a linear function, by use of Pearson's correlation; Sanpei et al. 1996). As in our series, a wide range of onset age was observed in Japanese patients with repeats between 35 to 45 (ages 20–60 years), while none of those reaching a threshold of 45 repeats or more had onset at >25 years of age. Furthermore, in both populations the variance of age at onset with changes of repeat size between 35 to 45 is significantly greater than the variance in age at onset observed with repeat sizes ≥45 (analysis not shown). Although we cannot determine at this point whether this represents a threshold effect or a continuously diminishing function, the decrease in variability in age at onset as a function of repeat size suggests that the genetic and epigenetic factors that modify age at onset exert significantly less influence as repeat size exceeds 45. While the mechanism underlying this effect is currently unknown, understanding its biologic basis may provide important clues as to the pathogenic mechanism of expanded SCA2 CAG repeats.

In some SCA2 kindreds, paternal transmission appears to lead to greater repeat instability and anticipation. For example, in the Italian FS kindred and several Martiniquan kindreds, there was no significant difference in age at onset between paternal and maternal transmissions, although both kindreds show marked an-

ticipation (Pulst et al. 1993; Lezin et al. 1996). In the current analysis, which excludes the Italian FS kindred, a difference between paternal and maternal transmissions is observed, as has been noted previously in Tunisian families (Belal et al. 1994). A similar degree of intergenerational difference in age at onset between maternal and paternal transmissions has been observed among Japanese SCA2 families (Sanpei et al. 1996). However, variability is large, and there is significant overlap between the two groups.

No allele sharing was found among the four families in whom it could be assessed, consistent with the hypothesis that the mutation occurred independently on different genetic backgrounds, without a single predisposing haplotype. Each kindred is of different ethnicity, so the possibility of founder chromosomes within populations of the same ethnic origin certainly remains. A previous study using markers less tightly linked to the SCA2 locus in a different population of families also showed no evidence for a founder effect (Gispert et al. 1993), and at least two disease haplotypes exist in Martiniquan SCA2 kindreds (Lezin et al. 1996), further supporting this notion. The absence of one founder or predisposing haplotype has also been demonstrated in SCA3/MJD, although founder haplotypes have been identified among certain specific ethnic groups in MJD (Stevanin et al. 1995).

Clinical Heterogeneity

The clinical heterogeneity among the six kindreds testing positive for SCA2 was striking, encompassing the MJD phenotype, typical dominant ataxia, and ataxia with prominent dementia and rapid disease course. The etiology of this variation is unknown, but potential mechanisms include somatic mosaicism, environmental influences, repeat size, or the effect of genetic background. The latter possibility is intriguing, given the apparent clustering of dementia in kindreds of African origin. The effect of repeat size can not be discounted, however, because our African American family with dementia had the largest average repeat size. More kindreds need to be evaluated to separate familial effects from the influence of repeat length.

Although the clinical findings in the SCA2 families described here largely concur with previous descriptions based on linkage analysis, some differences were found. Slowed saccades appeared to be a common early feature in most of the patients in our series (92%) but was less prevalent in previously reported SCA2 patients (Durr et al. 1995*a*). Chorea was also more common in our series, occurring in 38% of patients from three of five families, and has also been described in Japanese kindreds (Sasaki 1993). It is notable that each of our three families with chorea is small, with few living affected members available for examination. Perhaps chorea, as has been noted for dementia (Durr et al. 1995*b*), is associated with early onset and a more rapid course in SCA2, similar to the dystonic type I MJD (Sudarsky and Coutinho 1995). We found no clear association between the length of the SCA2 CAG repeat and chorea, but the number of patients analyzed was small.

Dementia is rarely observed in SCA1 and SCA3/MJD. Severe dementia was a conspicuous characteristic of patients from one SCA2 pedigree in this study, and dementia has been reported in $\leq$34% of members of Martiniquan kindreds with SCA2 (Cancel 1995*b;* Durr et al. 1995*b*). The presence of dementia in a subset of SCA2 families indicates that, when dementia is a prominent feature in a patient with ADCA, SCA2 is more likely to be the cause than is either SCA1 and SCA3. Other clinical features are unlikely to aid significantly in the differentiation of SCA2 from the other ADCAs (Durr et al. 1993, 1995*a;* Cancel et al. 1995*b*).

Knowledge of the mutational basis of SCA2 and the other ADCAs promises to simplify diagnosis and classification of the ataxias. However, it also emphasizes the mystery inherent in how the same mutation can lead to diverse phenotypes. It is striking that a CAG repeat expansion underlies all of the dominant ataxias thus far identified. It appears that this mechanism could account for the majority, if not all, of the ADCAs. Although the ADCAs have many features in common with other diseases linked to CAG expansions, such as HD and DRPLA, the clinical and pathological heterogeneity observed in all of the ADCAs contrasts with the relatively homogeneous pathology and clinical syndrome observed within families afflicted with HD. Whether this is due to the level of protein expression, the local protein milieu surrounding the repeat, or the expression of differentially interacting genes in specific cell types remains unknown. It is clear that understanding the genetic and epigenetic factors that modify the expression of the CAG repeat diseases is rapidly becoming a central issue for those studying degenerative neurological conditions.

Acknowledgments

The authors thank Dr. Jeffrey Gregg for his critical reading of the manuscript, Jan M. Hanley for statistical assistance, and Dr. Iscia Cendes-Lopez for control SCA1 and SCA3 DNAs. The authors also wish to thank the families followed in the UCLA Ataxia Clinic for their participation, as well as the staff of the UCLA Neurological Services for their continuing help and support. This work was supported by NIH grants NS011849 (to D.H.G.), NS33123-012A (to S.M.P.), the Carmen and Louis Warschaw Endowment fund for Neurology (to S.M.P.), and the Ruth and Lawrence Harvey Endowment fund for the neurosciences (to S.M.P.).

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